

Exposure to biological agents in school environments in Hungary with special focus on fungi

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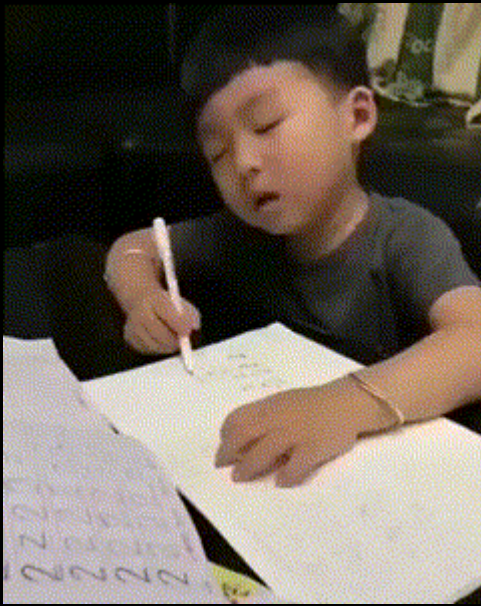
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Children spend ~8 hours
in school buildings/day

Vulnerable age group

- Immune system
- Sensibility for respiratory illnesses (asthma, allergy,...)





sinphonie

Schools Indoor Pollution and Health: Observatory Network in Europe



6 schools/Hungary
3 classrooms/school
2 sampling/classrooms,
4 week between samplings

Partner Countries



As you can see on the map twenty-one EU Member States, Albania, Bosnia and Herzegovina, Norway and Serbia are in the project. The thirty-eight partners from the twenty five countries represent the European network of the environment and health observatory.



16 schools/Hungary

1 classrooms/school

2 sampling/classrooms

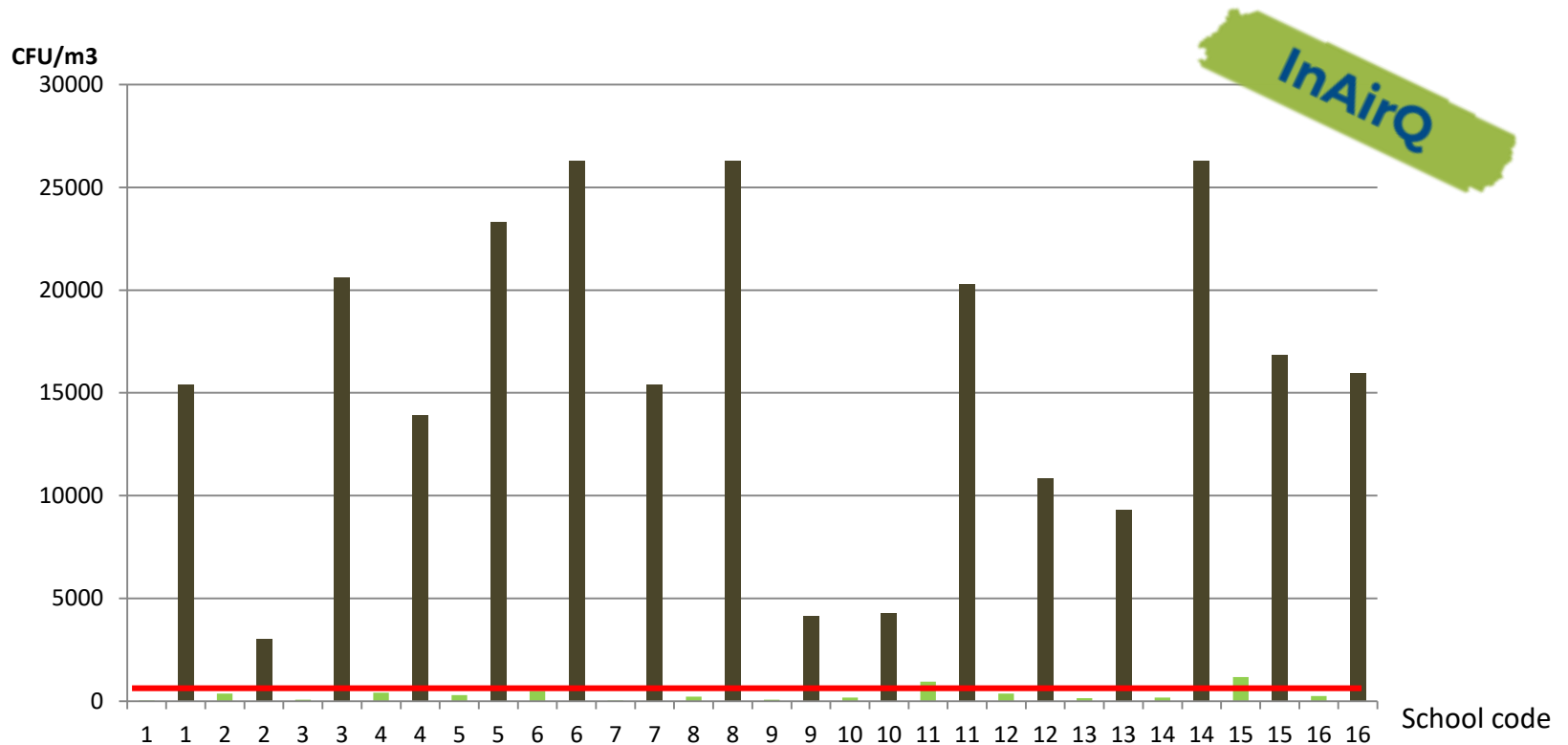


Materials & Methods

- Sampling sites
 - 11 towns in Hungary, 22 primary schools, 34 classrooms + outdoor reference
 - Age group of children: 8-10 ys
- Sampling method
 - Air samples (100 l) were collected with a single-stage Andersen-type sampler
 - The samples were taken during the lessons with closed windows and doors
- Sample processing
 - Bacteria have been incubated on Blood Agar at 37 °C for 3 days
 - Allergenic fungi have been incubated on Malt Extract Agar with 2% chloramphenicol at 25 °C for 5 days
 - morphological characterization

Results

Airborne bacteria



Concentration of bacteria in the air samples collected from classrooms (brown) and outdoors (green). Red line: threshold (500 CFU/m³, if above outdoor level)

Surprisingly high concentration!



No pathogenic bacteria
were detected.

Mostly *Bacillus* and *Micrococcus* spp.

Particles falling from the human body
1.000.000 particulate/h ($>0.5 \mu\text{m}$)
mostly bacteria

‘personal microbial cloud’
(Meadow et al. 2015)



Endotoxins

lipopolysaccharides, the major components of the outer membrane of **Gram-negative bacteria**.

Released from dead bacteria.

Symptoms:

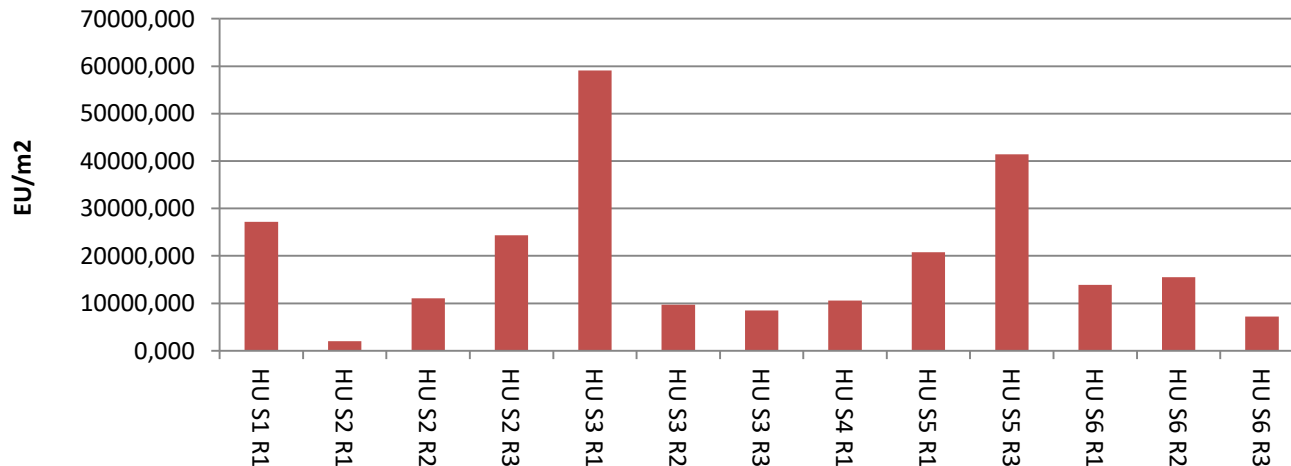
Fever, accelerated breathing and low blood pressure

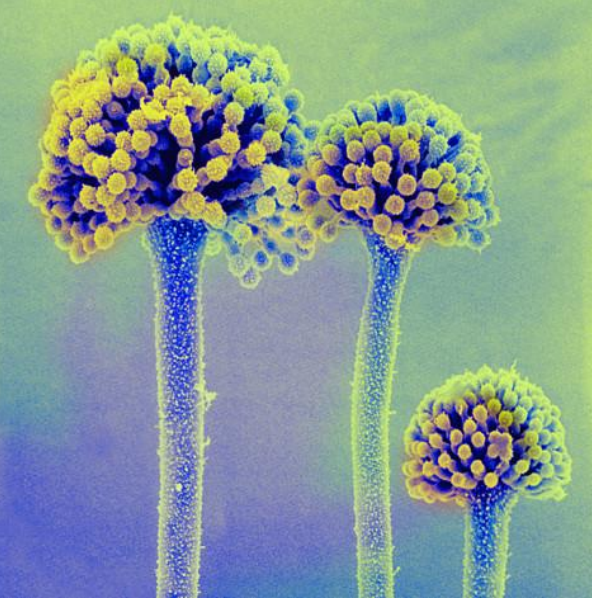
The effect is increased in the presence of tobacco smoke and NO_2

Positive effects?!

It can be protective against atopic / asthmatic diseases

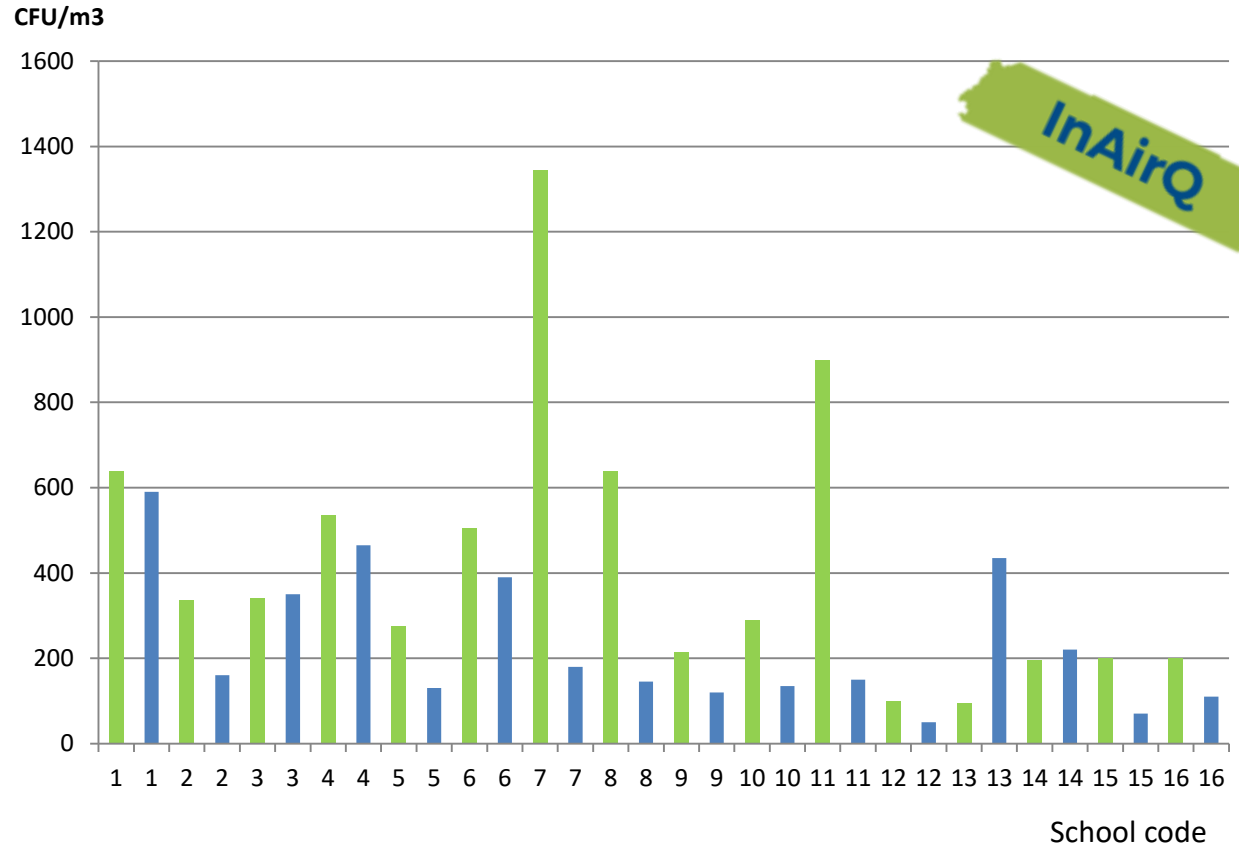
e.g. in airway remodeling (Liu 2002)





Fungi

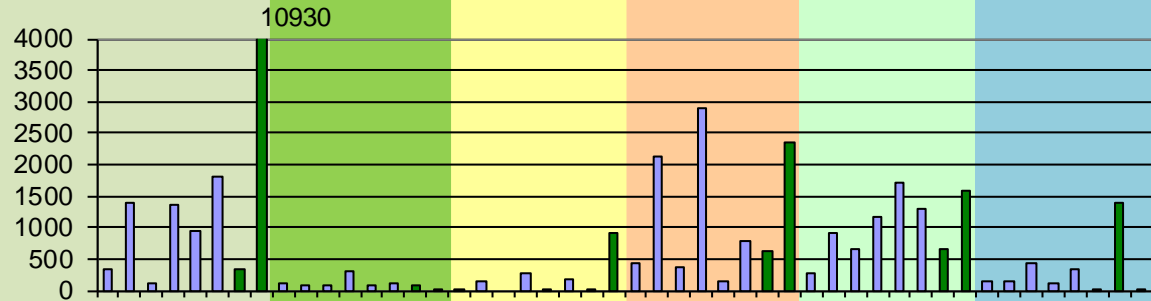
Total concentration of fungi
in the air samples collected from
classrooms (blue) and outdoors (green).



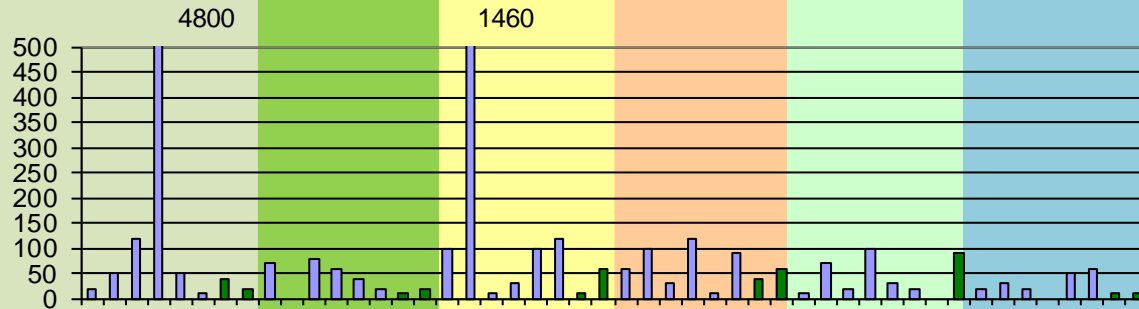
Concentration of **common** airborne fungi collected from classrooms (**blue**) and outdoors (**green**).

CFU/m³

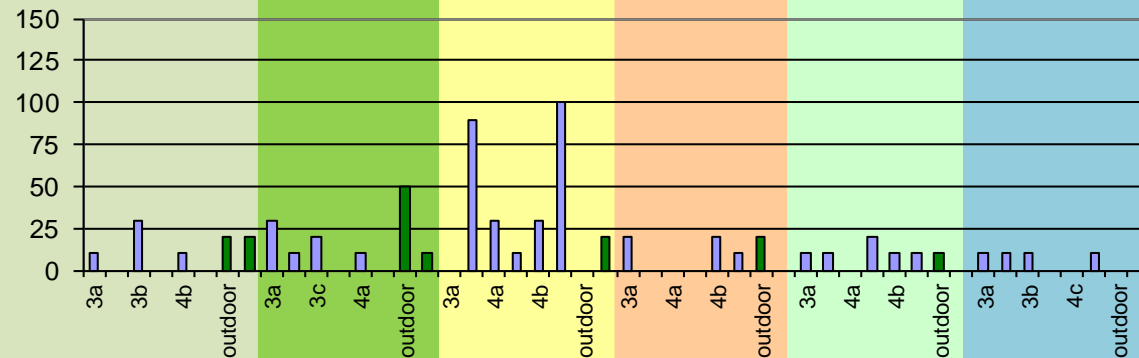
Cladosporium spp.



Penicillium spp.



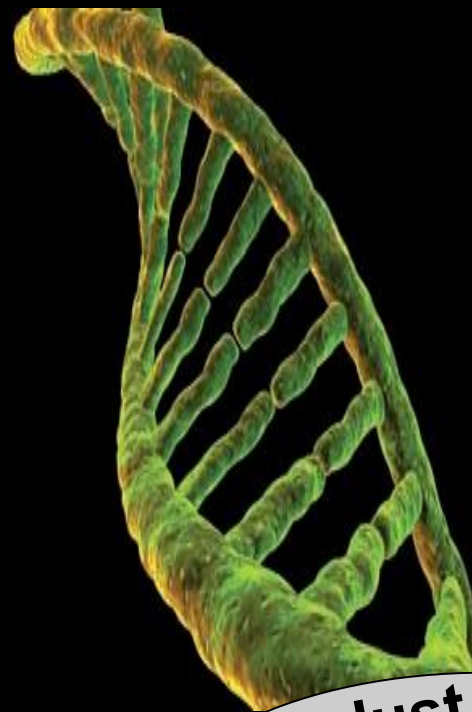
Aspergillus spp.





air

culture

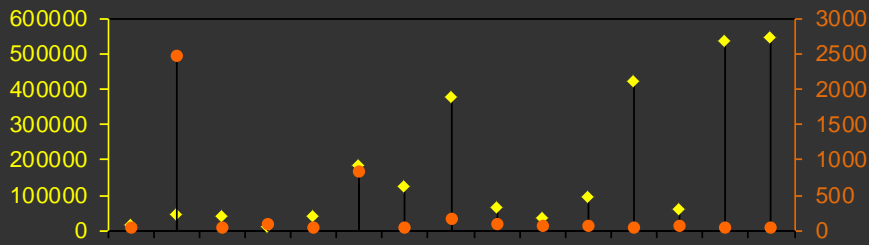


dust

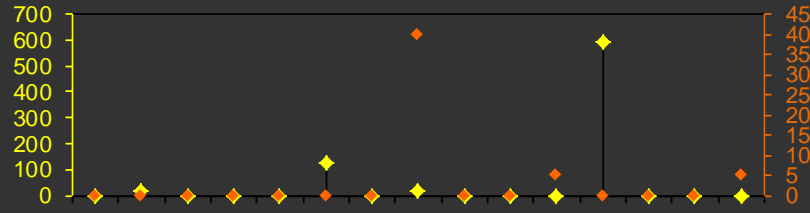
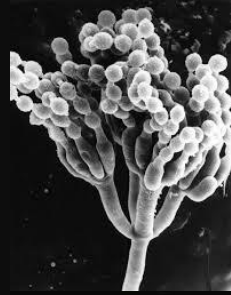
qPCR



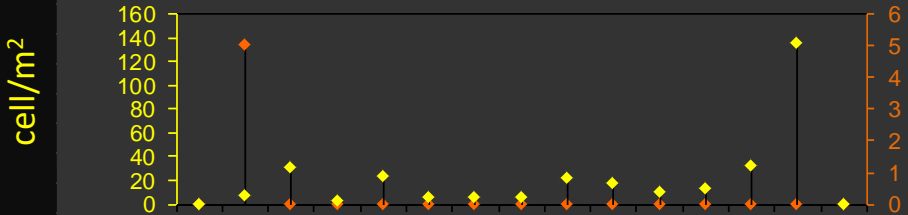
In collaboration with
Uppsala University, Swenden - Dan Norbäck
National Institute for Health and Welfare, Finland Martin Täubel



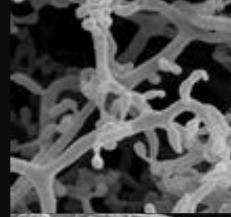
Aspergillus+Penicillium spp.



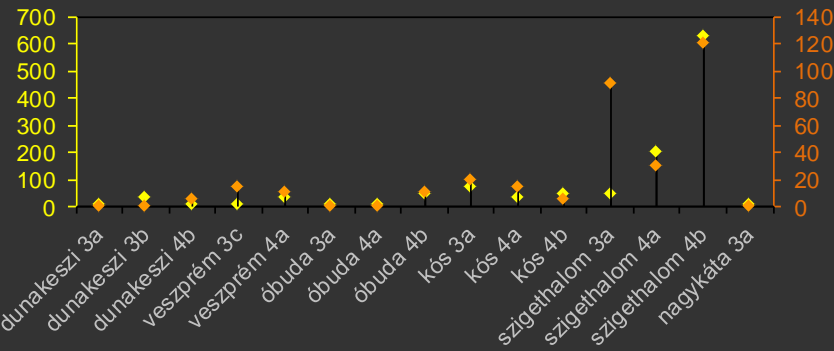
Aspergillus versicolor



Trichoderma spp./ *T. viridis*



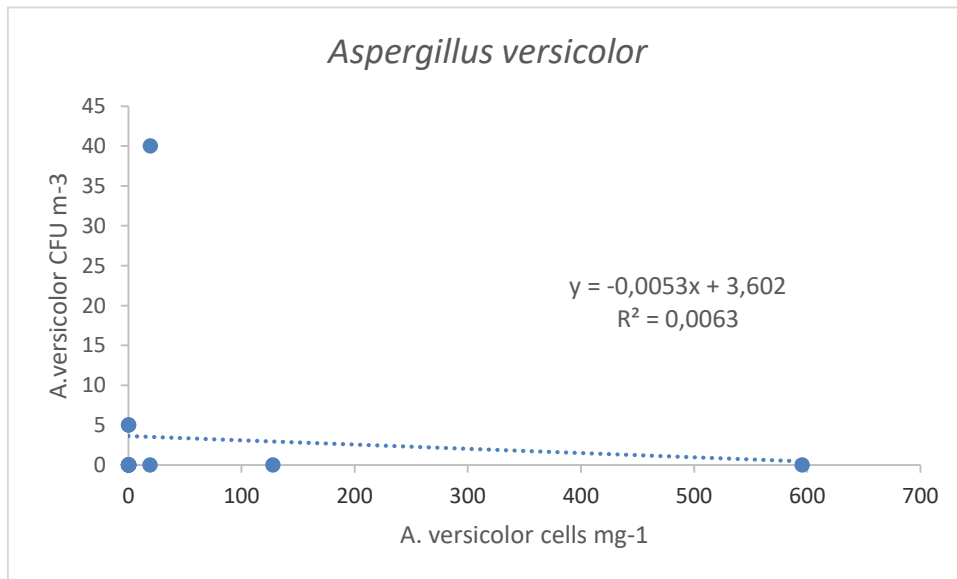
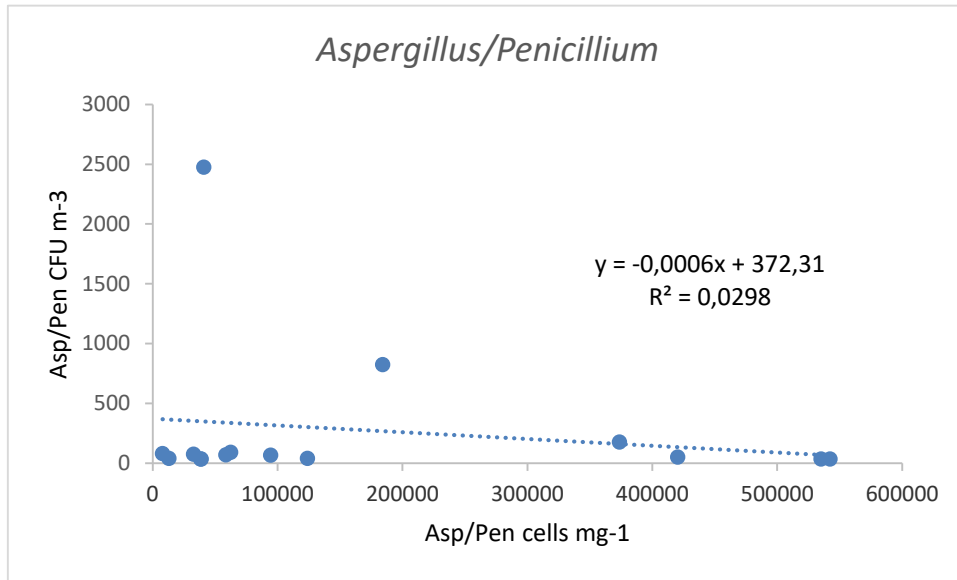
Cladosporium herbarum



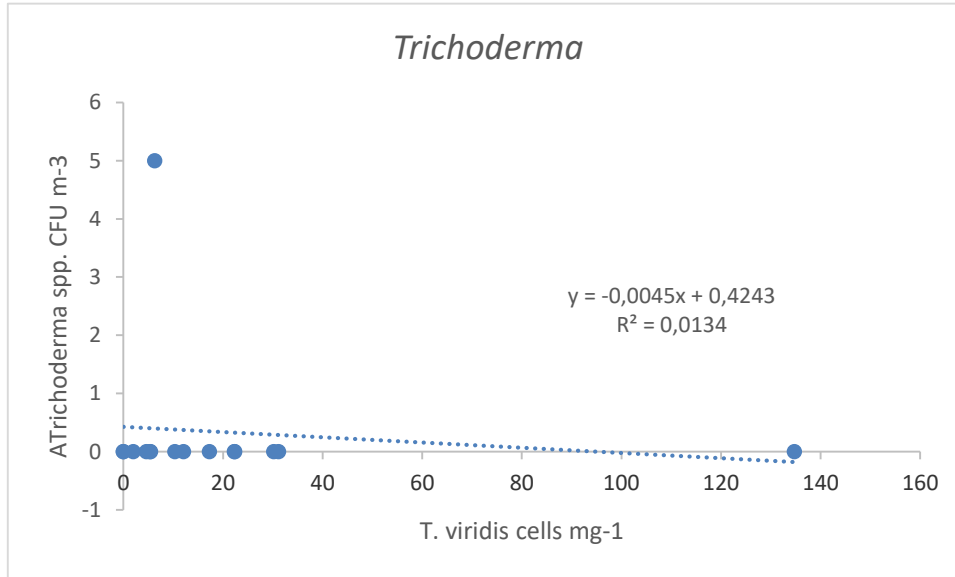
Alternaria spp./ *A. alternata*



Common indoor fungi: no correlation between concentration of fungi in settled dust and air

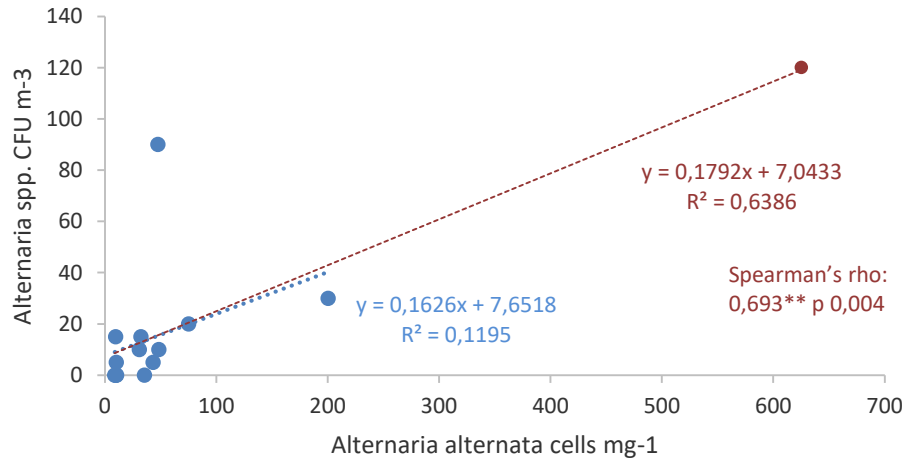


A soilborne fungus: no correlation between concentration of fungi in settled dust and air

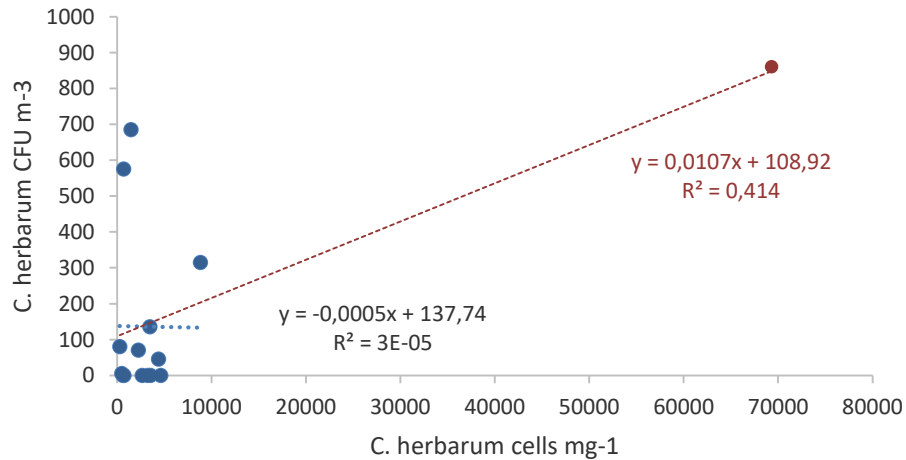


Common outdoor fungi: correlation between concentration of fungi in settled dust and air

Alternaria

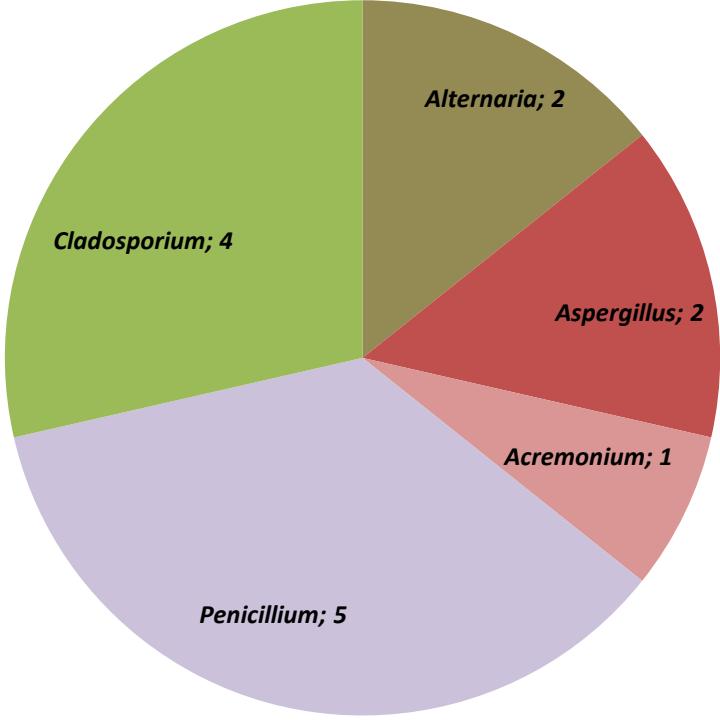


Cladosporium herbarum



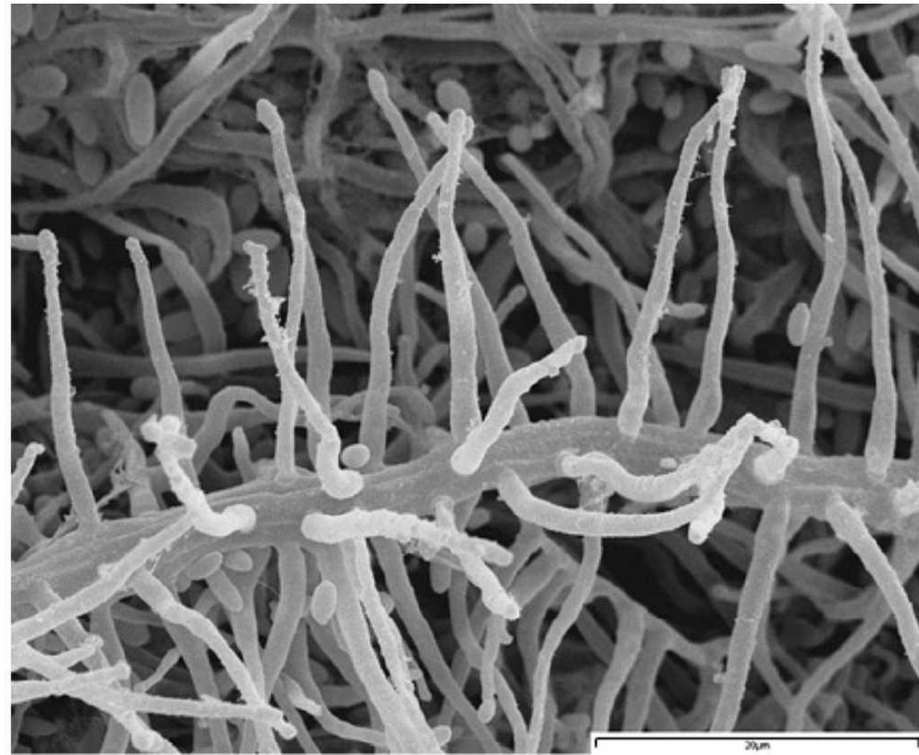
Airborne levels of moulds exceeded the threshold value in 41% (9) schools 38% (13) classrooms.

Number of cases when the concentration of fungal taxa exceeded the threshold level:



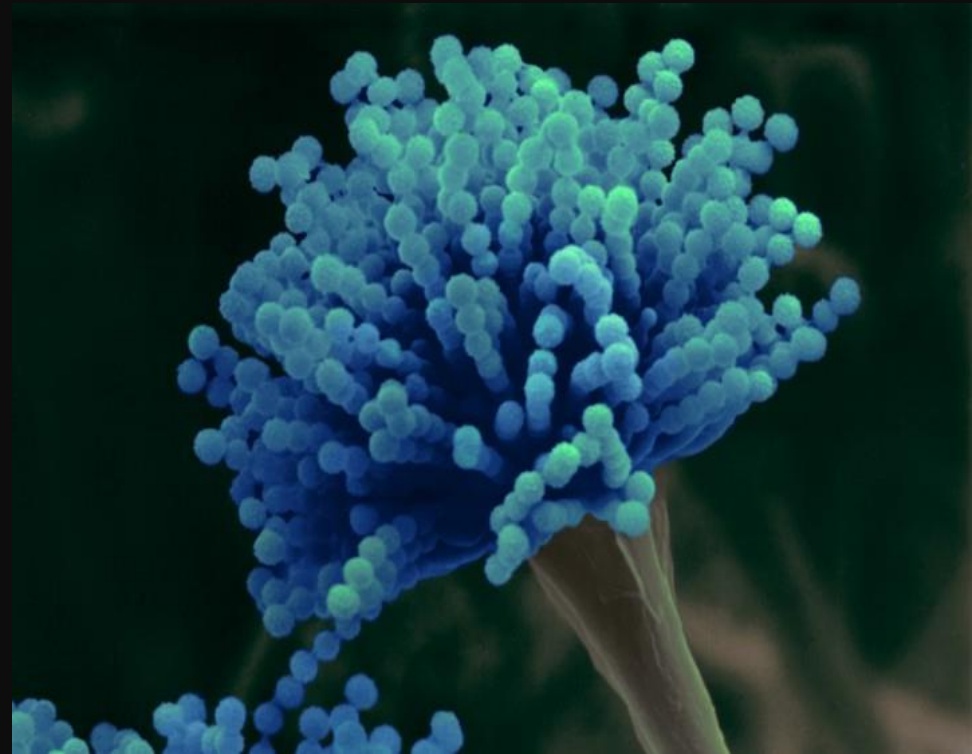
Threshold levels were defined as the concentration (colony forming units/m³) higher than the corresponding outdoor concentration of a given fungal sp. by 50.

Although *Cladosporium* species are common outdoor fungi, they are able to grow in areas of condensation, where they are frequently associated with *Acremonium* spp.

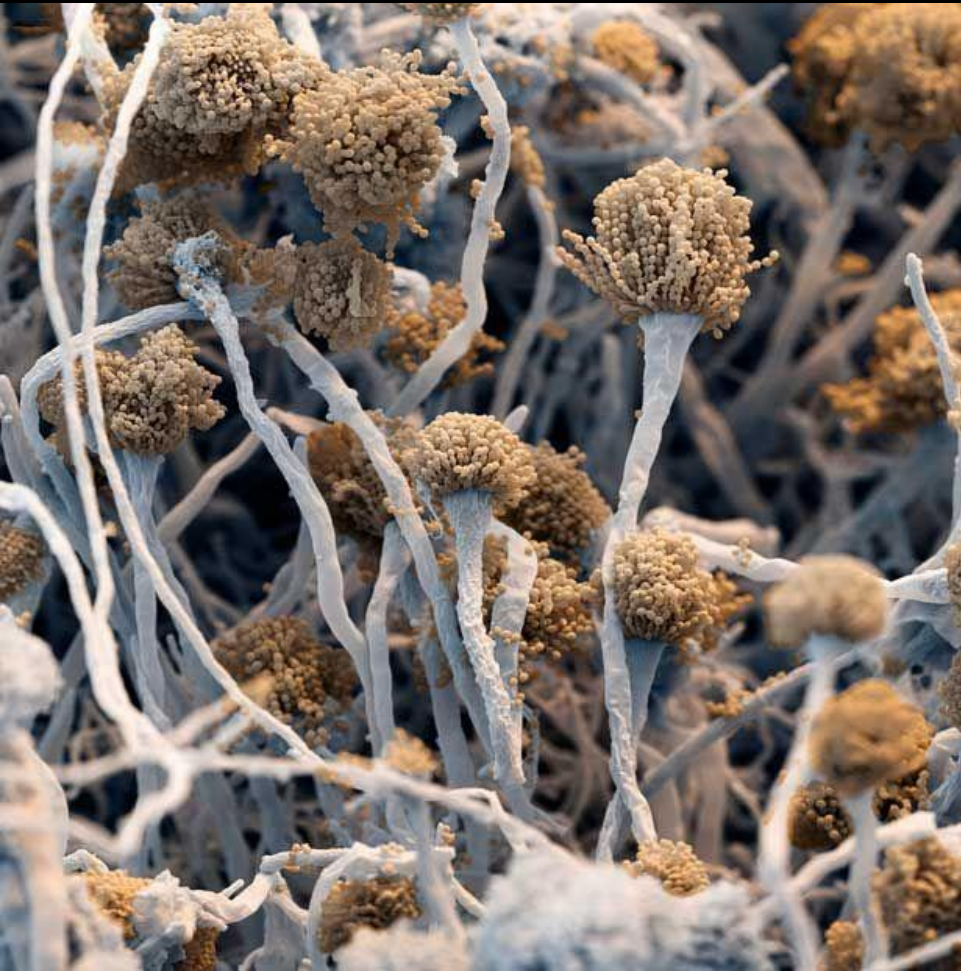


Aspergillus section *Versicolores*
(*A. versicolor*, *A. sydowii*)
are frequent in damp buildings.

Their high concentration could be
regarded as an indicator of fungal growth
in schools.



Aspergillus fumigatus
in composts



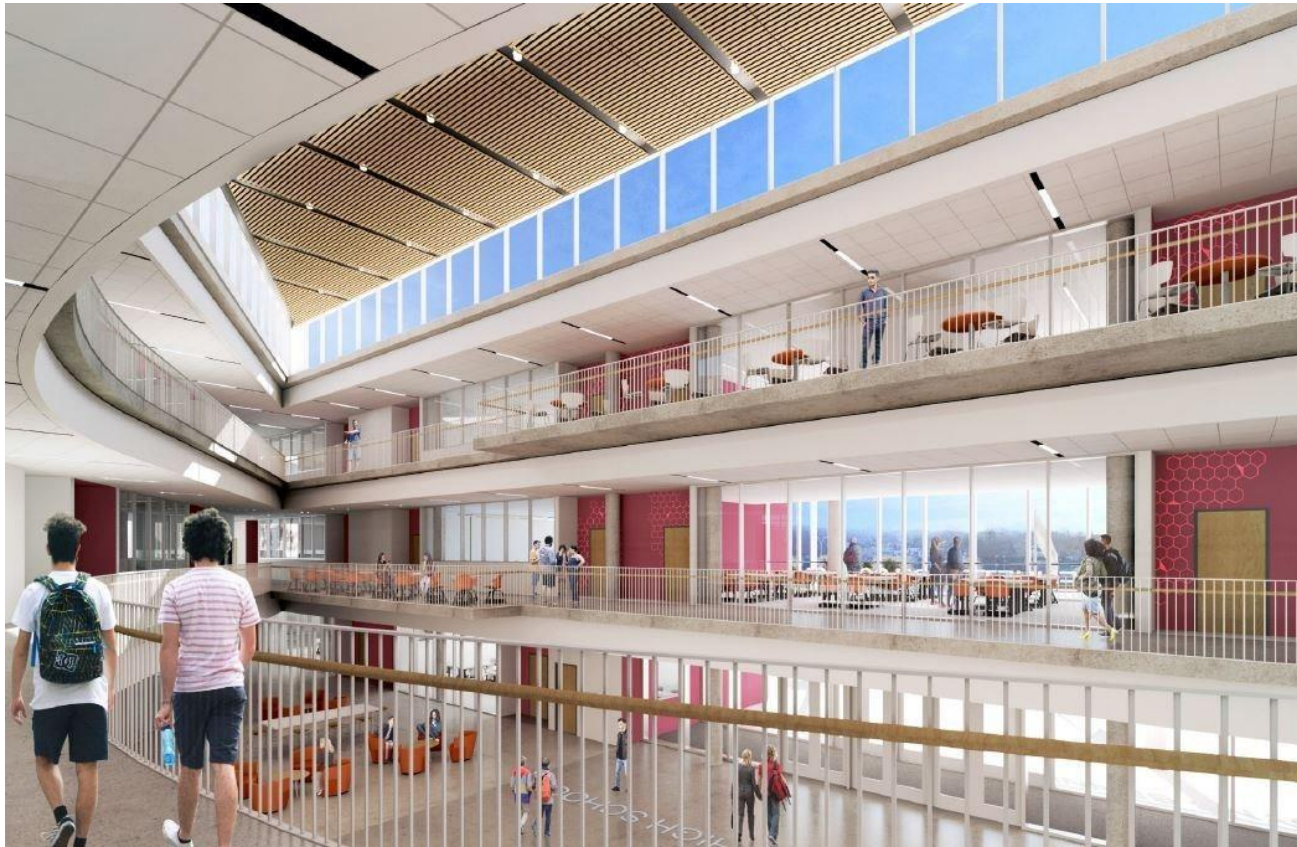
Penicillium spp.

common indoor and food-borne fungi.

Remnants of mouldy foods in schoolbags might be a major source of this fungus in classrooms.





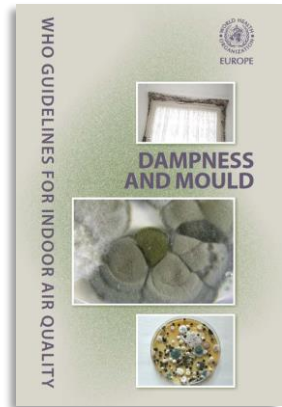


Modern buildings

dropped ceiling
airducts
gypsum board walls...



hidden mold



**Visual inspection
(suggested by guidelines)
is not effective anymore.**

**But...
sampling-based
detection methods
have limitations
-expensive,
-time-consuming
-needs expertise
-etc...**



Conclusion

- It is important to improve microbial air quality in schools.
- Modern buildings are new challenge for microbiologists.
- Research should focus on
 - the development of a new, simple method to detect hidden mold
 - risk assessment of mold exposure
 - following new technologies in architecture and keep environmental health guidelines updated

Thank you for your attention!



European Union

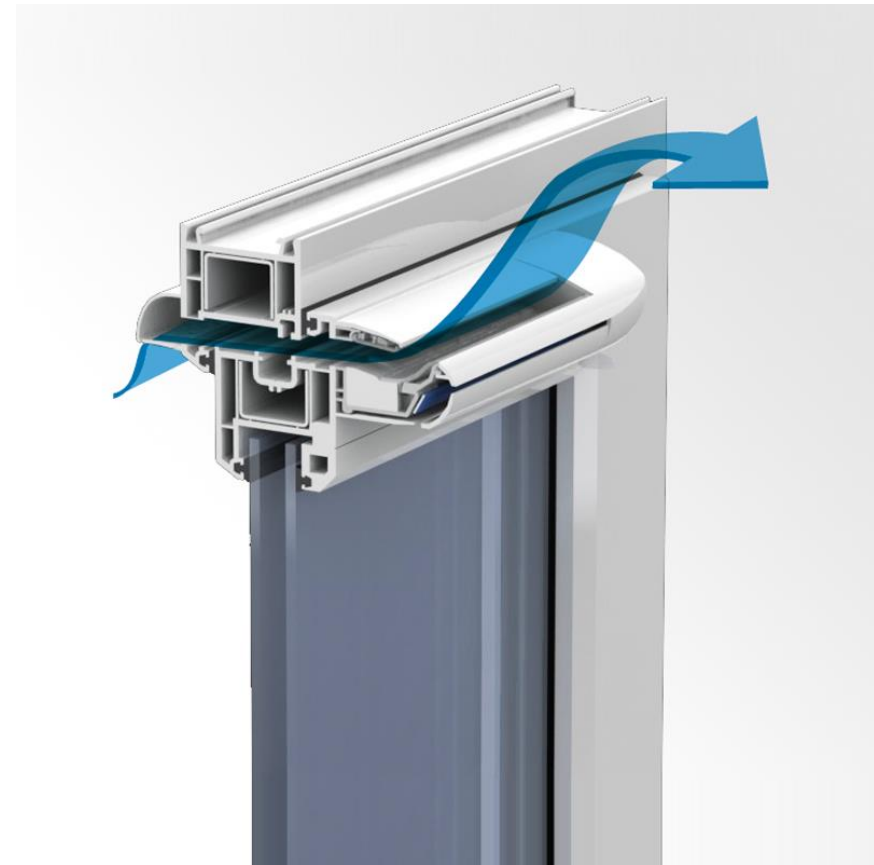


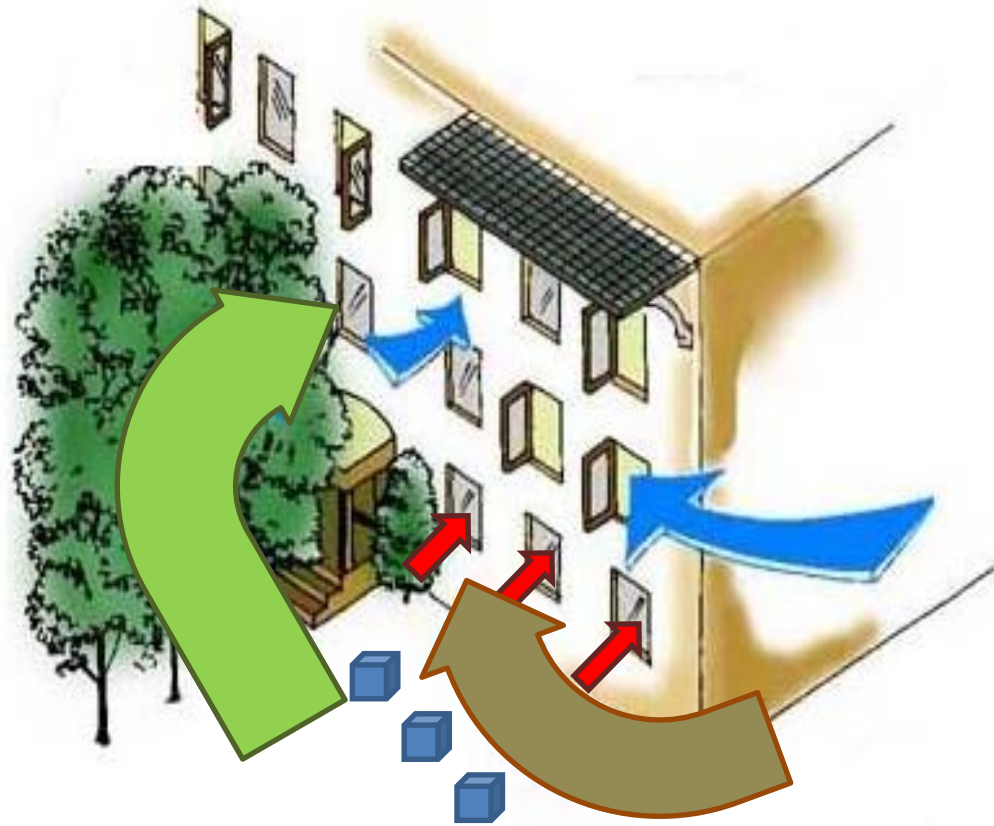
Interreg
CENTRAL EUROPE

InAirQ

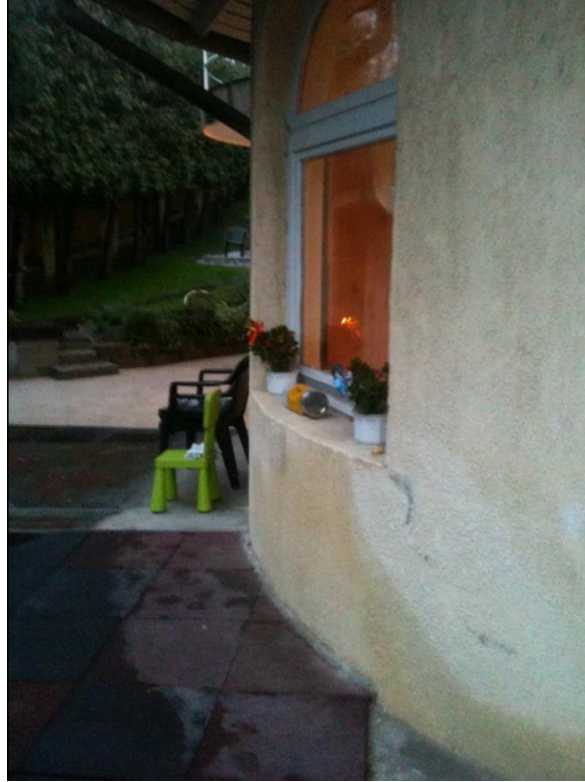
Acknowledgements: This work was implemented within the projects SINPHONIE (Schools Indoor Pollution and Health, initiated and funded by the European Parliament) funded by DG Sanco, Health and Consumer Protection Directorate and InAirQ (Transnational adaption actions for integrated indoor air quality management) funded by Interreg CENTRAL EUROPE.

Window vent (air intake)





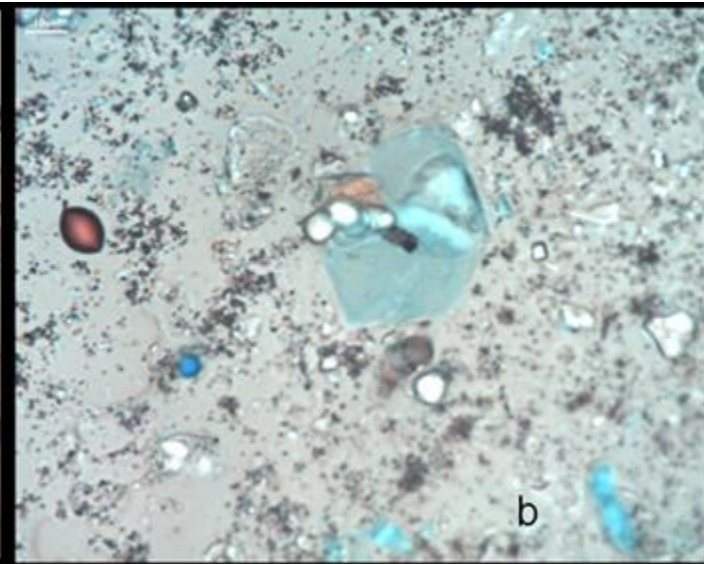
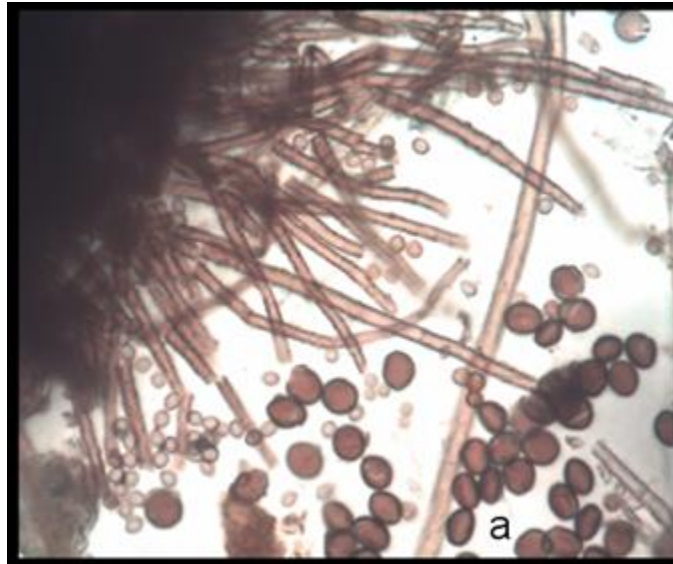
4. eset



A csoportszoba fala az ereszcatorna rossz bekötése és az alap mentén történő beázások miatt vizesedik.

A tapétarétegek alatt *Chaetomium* gomba telepei és háziporatkák.

A spórák kiszóródtak.



Penészgombák

(Hyphomycetes, Zygomycetes):

80 faj!

szenzitizálás

allergiás rhinitis

atópiás dermatitis

asztma

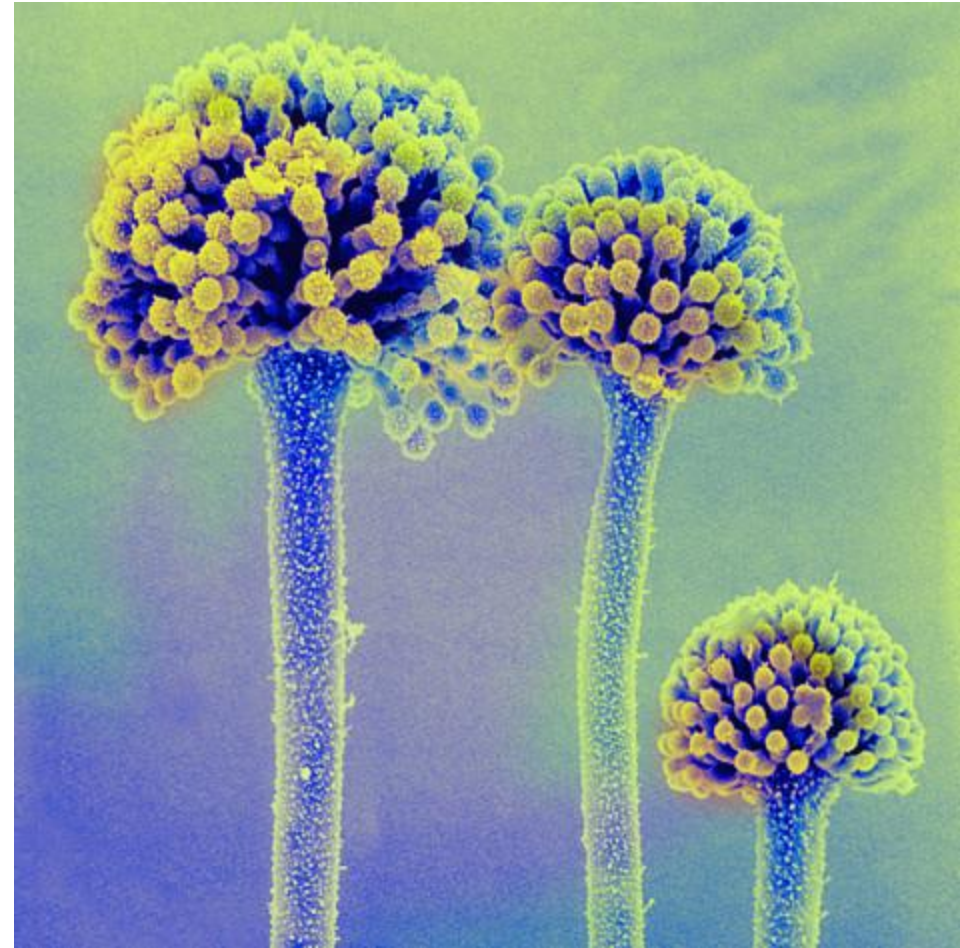
tüdőmikózis, aspergilloma,

hyperszenzitív tüdőgyulladás

humidifier fever,

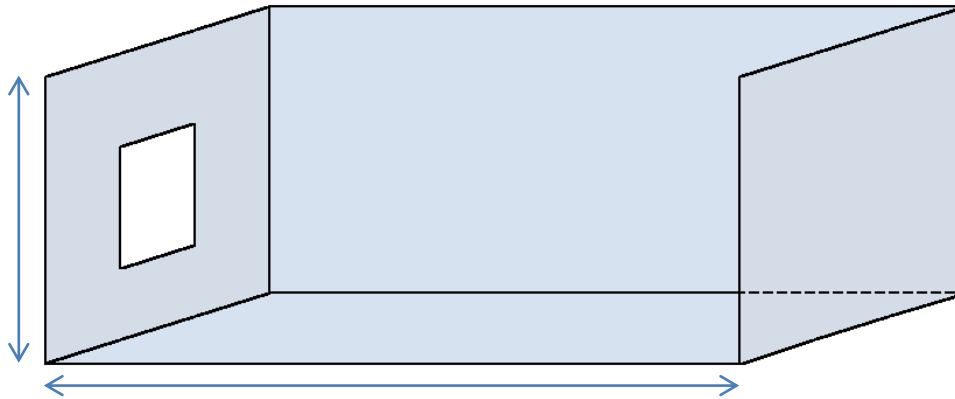
SBS

BRI

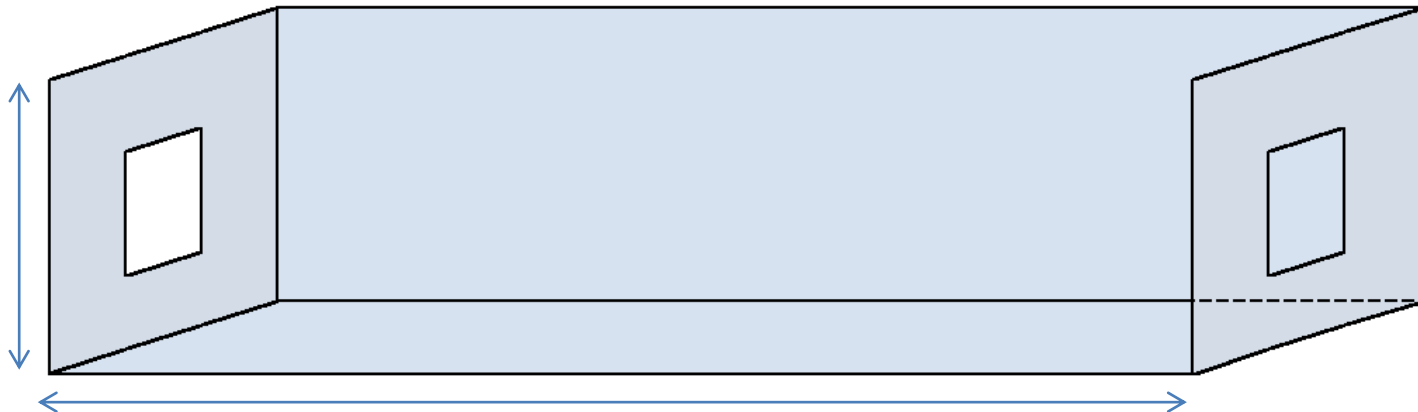


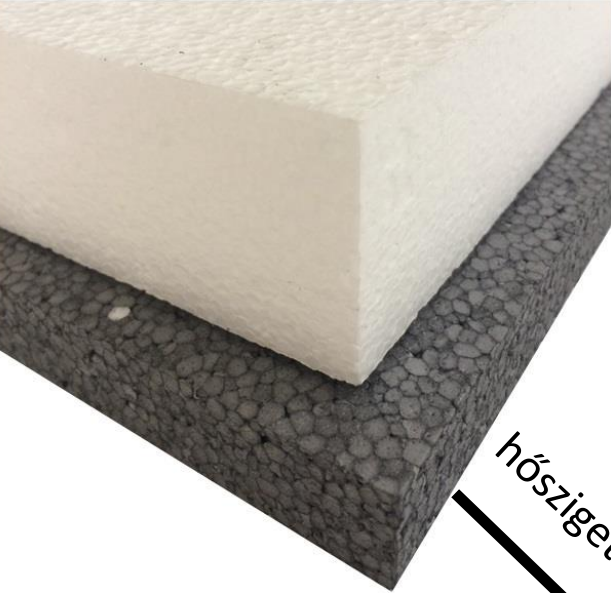
A természetes szellőzés akkor hatékony, ha

egyoldali szellőzésnél a tér mélysége a belmagasság 2,5-szeresénél nem nagyobb



átszellőzésnél a tér mélysége a belmagasság 5-szörösénél nem nagyobb.





hőszigetelés



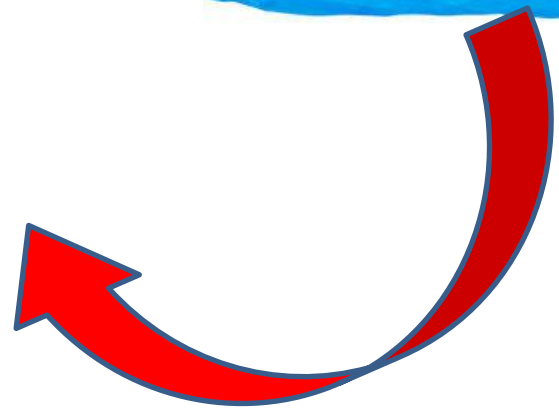
műanyagalapú falfesték



légtömör nyílászárók



penész



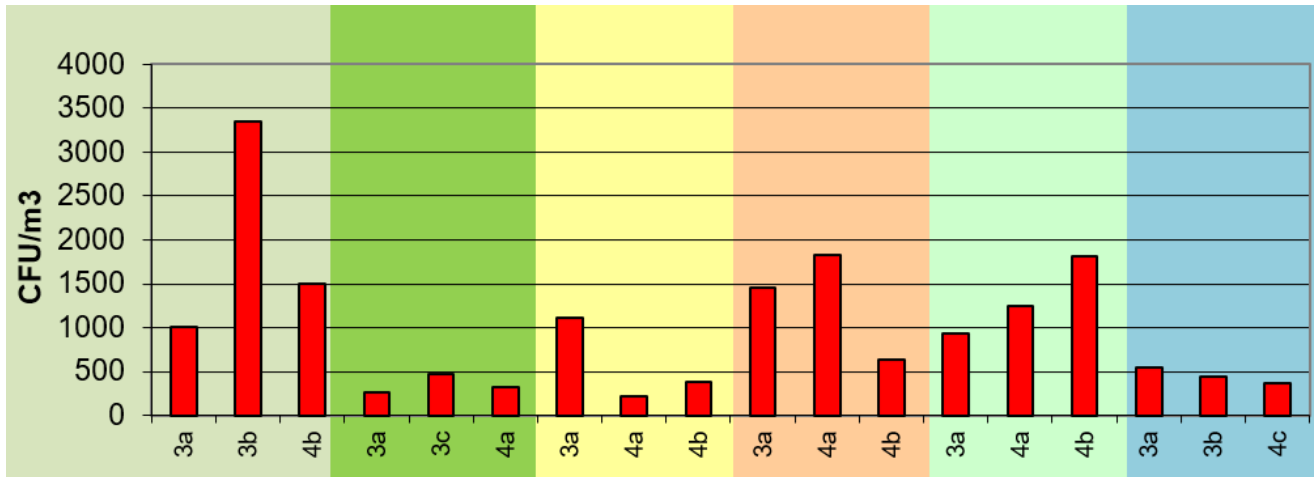
3. eset

- Padlásajtó nyílik a lakótérbe
- A padlásról MMVF törmelék szóródik,
- kimutatható a gyerekszobában

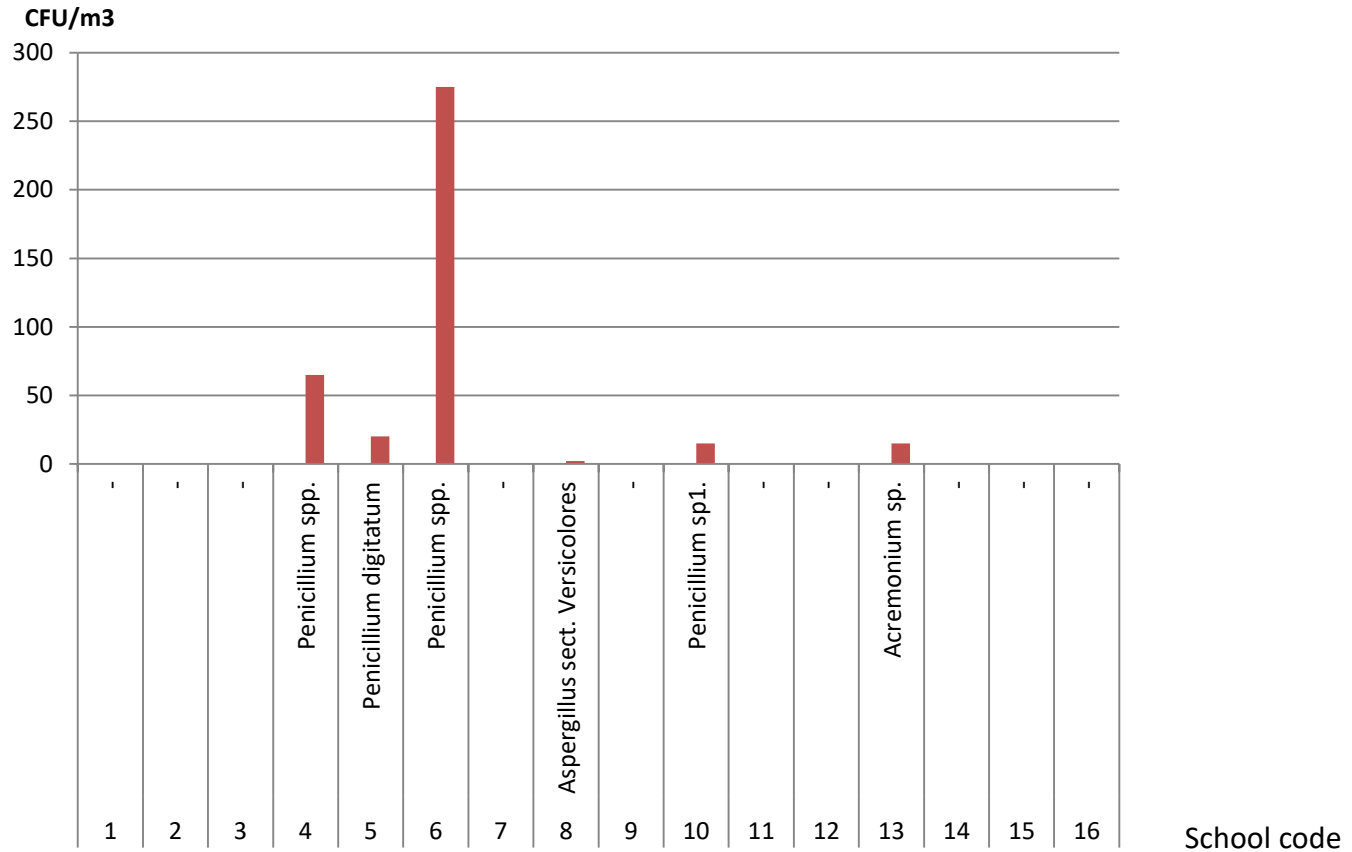


Reményné Nagy Z, Magyar D, Varró M, Nagy L,
Mácsik A, Szentmihályi R, Rudnai P, Beregszászi
T (2012) Iskolás gyerekek endotoxin-
terhelésének vizsgálata passzív pormintavételi
módszerrel. TOX'2012 konferencia, Hévíz,
2012. október 17-19.

Total concentration of airborne fungi in Hungarian schools



Fungal taxa having concentration above threshold level



Threshold levels were defined as the concentration (colony forming units/m³) higher than the corresponding outdoor concentration of a given fungal sp. by 50.

- Isolated fungal taxa:

- ***Acremonium* sp.**

- *Aspergillus* sp.

- *A. clavatus*

- *A. flavus*

- *A. sydowii*

- ***A. sect. Versicolores***

- *Beauveria* sp.

- *Bipolaris* sp.

- *Cladosporium* sp.

- *Eurotium* sp.

- ***Penicillium* sp.**

- ***P. digitatum***

- *Phoma* sp.

- *Rhodotorula* sp.

- *Sporothrix* sp.

- *Talaromyces* sp.

- *Trichotecium roseum*

- *Ulocladium* sp.

Long-term exposure to indoor air pollution could lead to respiratory diseases, such as allergy and asthma. Children aged 6 to 14 represent one of the most sensitive groups, spending 6-8 h daily in classrooms. Air samples were collected in 22 primary schools (34 classrooms and outdoors) in 11 towns in Hungary with a single-stage Andersen device. Two samples per classroom were taken during the lessons with closed windows and doors onto malt extract agar with 10% chloramphenicol and incubated at 25 °C for 5 days. Threshold levels were defined as the concentration (colony forming units/m³) higher than the corresponding outdoor concentration of a given fungal sp. by 50. Sporulating filamentous fungi were identified at the genus level with a Carl Zeiss Jenaval light microscope at 300×. Airborne levels of moulds exceeded the threshold value in 9 schools (13 classrooms). *Penicillium* (36%), *Cladosporium* (29%), *Alternaria* (14%), *Aspergillus* (14%) and *Acremonium* (7%) spp. reached high concentrations. Many species of *Penicillium* are common indoors and are food-borne fungi. Remnants of mouldy foods in schoolbags might be a major source of this fungus in classrooms. *Alternaria* and *Cladosporium* are common outdoor fungi, but the latter one often grows in areas of condensation, where it is frequently associated with *Acremonium* spp. *Aspergillus* spp. (especially spp. of the section *Versicolores*) are frequent in damp buildings. Their high concentration could be regarded as an indicator of fungal growth in schools. The above-mentioned fungi can trigger respiratory diseases such as allergy and asthma. Other biological agents, such as bacteria in Hungarian schools are also reviewed. Based on these results it is important to improve microbial air quality in schools.



LAL- Limulus amoebocyte lysate teszt